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First description of three ciliates (Ciliophora: Stichotrichia) from Korea

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We firstly report three stichotrichs, *Metaurostylopsis cheni* Chen, Huang & Song, 2010, *Gonostomum strenuum* (Engelmann, 1862) Sterki, 1878, and *Lamtostyla granulifera* Foissner, 1997, collected in South Korea. We investigated these ciliates based on observations of live and protargol-impregnated specimens.

Keywords: *Gonostomum*, Korea, *Lamtostyla*, *Metaurostylopsis*, Stichotrichs

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Introduction

The genus *Metaurostylopsis* was established by Song *et al*. (2001). The characteristics of this genus are as follows: clearly differentiated frontal, buccal, and transverse cirri; frontoterminal cirral row present; caudal cirri absent; and more than one left and right marginal row (Song *et al*., 2001). The genus *Metaurostylopsis* includes six species (Song *et al*., 2001; Berger, 2006; Shao *et al*., 2008; Chen *et al*., 2011; Jung *et al*., 2011).

The genus *Gonostomum*, the type genus of the family Gonostomatidae, was established by Sterki (1878), and it includes about 10 ciliate species, including questionable synonymous species (Foissner *et al*., 2002; Berger, 2011; Bharti *et al*., 2015). The *Gonostomum* has the features as follows: shortened undulating membrane; mostly three bipolar dorsal kineties; caudal cirri present; and dorsomarginal kineties and kinety fragmentation lacking (Berger, 2011).

The genus *Lamtostyla* was established by Buitkamp (1977), and it comprises 12 species (Berger, 2008). Several features that are present in the *Lamtostyla* as follows: usually short median cirral row terminated ahead of mid-body; three bipolar dorsal kineties; caudal cirri lacking; and terrestrial habitat (Berger, 2008).

Herein, we describe the three stichotrichs - *Metaurostylopsis cheni* Chen, Huang and Song, 2010, *Gonostomum strenuum* (Engelmann, 1862) Sterki, 1878, and *Lamtostyla granulifera* Foissner, 1997 - all of which are new to Korea. Our descriptions are based on observations of live and protargol-impregnated specimens.

Materials and Methods

Sample site and morphological identification

The marine ciliate, *Metaurostylopsis cheni* was isolated from Mokpo Harbor and Yongyu-dong, Incheon, Korea. Populations were cultured in Petri dishes at room temperature with rice grains to enrich the bacterial growth that served as a food resource. *Gonostomum strenuum* and *Lamtostyla granulifera* were collected from soil samples from Jeju-do Province and Gangwon-do Province, respectively. The soil samples were transferred into Petri dishes, and mineral water was added, and the samples were allowed to stand at room temperature for more than 6 hours.

A single clone of *Metaurostylopsis cheni*, and raw cultures of *Gonostomum strenuum* and *Lamtostyla granulifera* were investigated. Live and protargol-impregnated specimens were observed using a stereo microscope (SZH10; Olympus, Japan) and a light microscope (DM2500; Leica, Wetzlar, Germany) at magnifications of ×50 to ×1000. Protargol impregnation was performed according to Foissner (1991). Classification and terminology follows Berger (2006; 2008; 2011).

Systematic Accounts

Order Urostylida Jankowski, 1979

Table 1. Morphometric data on *Metaurostylopsis cheni* (upper line, Korean population collected from Mokpo, this study; lower line, Chinese population from Chen *et al*., 2011).

Characteristics[a]	Mean	SD	CV	Min	Max	n
Body, length	68.9	7.59	11.0	55	83	20
	81.6	8.59	10.5	63	102	25
Body, width	28.7	3.99	13.9	23	37	20
	36.3	8.02	22.1	21	52	25
Adoral zone of membranelles, length	28.1	3.85	13.7	22	40	20
	29.7	1.97	6.7	26	32	25
Adoral zone of membranelles, number	27.0	2.26	8.4	23	31	20
	23.4	1.32	5.7	21	26	25
Frontal cirri, number (including III/2)	4.0	0	0	4	4	20
	4.0	0	0	4	4	25
Frontoterminal cirri, number	4.0	0.46	11.5	3	5	20
	4.0	0	0	4	4	25
Buccal cirri, number	1.0	0	0	1	1	20
	1.0	0	0	1	1	25
Midventral cirri, number	9.0	1.15	12.8	7	11	20
	6.8	1.07	15.6	5	9	25
Ventral cirri, number	3.9	0.49	12.7	3	5	20
	4.2	0.37	9.0	4	5	25
Transverse cirri, number	6.6	0.94	14.2	5	8	20
	6.2	0.69	11.2	5	8	25
Left marginal rows, number	3.2	0.37	11.6	3	4	20
	3.1	0.33	10.6	3	4	25
Left marginal row 1, number of cirri	17.4	4.50	25.9	4	23	20
	15.9	3.71	23.4	7	25	25
Left marginal row 2, number of cirri	20.6	3.14	15.3	15	28	20
	17.7	2.82	16.0	12	24	25
Left marginal row 3, number of cirri	19.8	2.88	14.6	15	24	20
	16.8	2.04	12.2	13	20	25
Left marginal row 4, number of cirri	17.7	–	–	15	20	3
	13.7	–	–	12	17	3
Right marginal rows	3.4	0.49	14.6	3	4	20
	3.0	0	0	3	3	25
Right marginal row 1, number of cirri	19.6	4.82	24.6	7	27	20
	18.3	2.46	13.5	15	24	25
Right marginal row 2, number of cirri	24.4	3.47	15.5	12	31	20
	19.6	2.36	12.0	16	24	25
Right marginal row 3, number of cirri	22.4	3.47	15.5	18	28	20
	17.3	2.53	14.6	13	21	25
Right marginal row 4, number of cirri	19.2	3.19	16.6	15	24	6
Dorsal kineties, number	3.0	0	0	3	3	20
	3.0	0	0	3	3	25
Macronuclear nodules, number	57.0	10.70	18.8	40	75	20
	40.0	8.40	21.0	30	65	25

[a]Data based on protargol-impregnated specimens.
CV = coefficient of variation (%); Max = maximum; Mean = average; Min = minimum; n = number of individual investigated; SD = standard deviation. All measurements in μm.

Family Urostylidae Bütschli, 1889
Genus *Metaurostylopsis* Song, Petz & Warren, 2001

Metaurostylopsis cheni Chen, Huang, & Song, 2010 (Table 1; Figs. 1, 2)

Diagnosis. Cell size 90-120 × 43-50 μm *in vivo*, flexible and yellow to greenish (Fig. 1A, 2A, C). Two types of cortical granules: large granules of oval shape, yellow to green in color, ca. 1.5 μm across, and distributed along with the cirral rows and dorsal kineties; small granules colorless and irregularly scattered, ca. 0.3 μm across (Fig. 2D, F). About 57 macronuclear nodules (Table 1). Three frontal and three to five frontoterminal cirri; buccal and III/2 cirrus present; five to eight transverse cirri; seven to eleven midventral pairs, additional three to five unpaired ventral cirri in a longitudinal row; three to four left and right marginal rows; three bipolar dorsal kine-

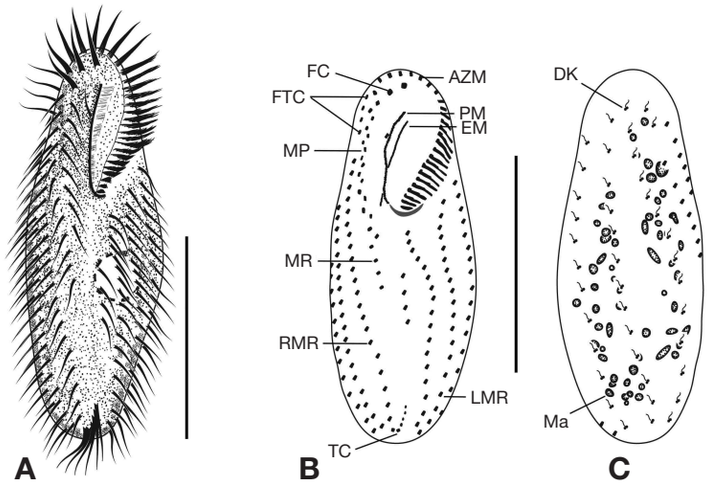


Fig. 1. Morphology of *Metaurostylopsis cheni* drawn from live (A) and protargol impregnated (B, C) specimens. A, ventral view of a typical specimen; B, C, cirral base in ventral and dorsal views; AZM, adoral zone of membranelles; BC, buccal cirrus; DK, dorsal kineties; EM, endoral membrane; FC, frontal cirri; FTC, frontoterminal cirri; LMR, left marginal row; Ma, macronuclear nodules; MVR, midventral rows; MP, midventral pairs; PM, paroral membrane; RMR, right marginal row. TC, transverse cirri. Scale bars: 50 μm.

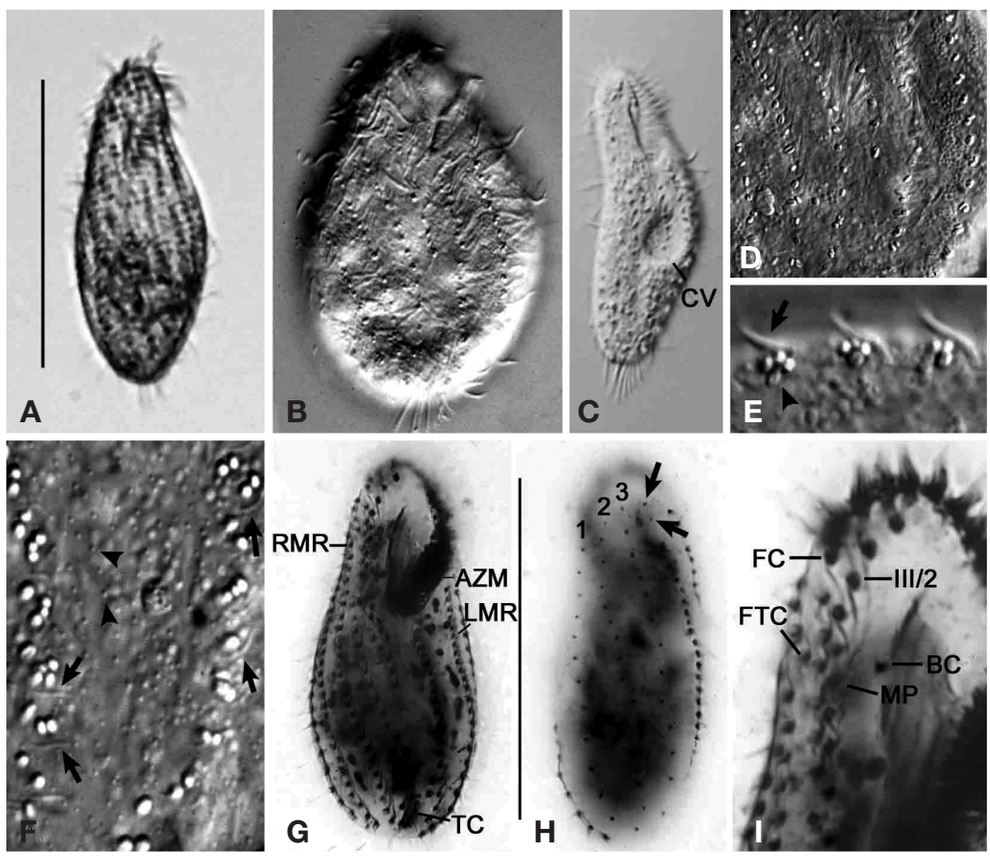


Fig. 2. Photomicrographs of *Metaurostylopsis cheni* from live (A-F) and protargol impregnated (G-I) specimen. A, B, C, ventral views; D, distribution of cortical granules on ventral surface; E, cirri (arrow) and large cortical granules (arrowhead) on ventral side; F, dorsal view, dorsal bristles (arrows) and small cortical granules (arrowheads); H, arrows indicate basal bodies; I, anterior region on ventral surface; III/2, III/2 cirrus; AZM, adoral zone of membranelles; BC, buccal cirrus; FC, frontal cirri; FTC, frontoterminal cirri; LMR, left marginal row; MP, midventral pairs; RMR, right marginal row; TC, transverse cirri; 1-3, dorsal kineties 1-3. Scale bars: 100 μm.

Table 2. Morphometric data on *Gonostomum strenuum* (upper line, Korean population, Jeju, from this study; lower line, Namibian population from Foissner *et al*., 2002).

Characteristics[a]	Mean	SD	SE	CV	Min	Max	n
Body, length	86.5	8.2	1.8	9.5	75.0	105.0	20
	81.5	7.8	2.2	9.6	72	98.0	13
Body, width	22.7	4.8	1.1	21.0	20.0	36.0	20
	30.0	2.9	0.8	9.6	26.0	36.0	13
Adoral zone of membranelles, length	39.6	3.1	0.7	7.7	35.0	45.0	20
	42.3	4.9	1.4	11.6	32.0	49.0	13
Anterior macronuclear nodule, length	15.5	2.2	0.5	14.0	13.0	19.0	20
	16.7	1.8	0.5	10.5	14.0	20.0	13
Anterior macronuclear nodule, width	5.2	0.9	0.2	17.2	4.0	6.0	20
	7.1	1.0	0.3	13.5	6.0	8.0	13
Micronuclei, number	2.4	0.7	0.2	28.4	2	4	20
	4.6	1.5	0.4	32.5	3	8	13
Adoral zone of membranelles, number	27.8	2.8	0.6	10.0	24	32	20
	27.9	2.4	0.7	8.6	24	31	13
Frontal cirri, number	3.0	0.0	0.0	0.0	3	3	20
	3.0	0.0	0.0	0.0	3	3	13
Buccal cirri, number	1.0	0.0	0.0	0.0	1	1	20
	1.0	0.0	0.0	0.0	1	1	13
Cirri in streak III, number[b]	2.8	0.4	0.1	14.7	2	3	20
	2.0	0.0	0.0	0.0	2	2	13
Cirri in streak IV, number	3.8	0.4	0.1	10.8	3	4	20
	3.5	0.7	0.2	18.7	3	5	13
Cirri in streak V, number[c]	7.6	0.7	0.2	9.0	7	9	20
	6.9	1.3	0.4	19.1	5	9	13
Frontoterminal cirri, number	4.9	0.9	0.2	17.4	4	6	20
	4.3	0.8	0.2	17.4	3	6	13
Pretransverse ventral cirri and transverse cirri, number	5.2	0.6	0.1	11.8	4	6	20
	4.7	1.1	0.3	23.6	2	6	13
Right marginal cirri, number	23.4	1.5	0.3	6.3	21	26	20
	20.5	3.5	1.0	17.2	16	27	13
Left marginal cirri, number	16.2	1.0	0.2	6.2	15	18	20
	14.9	1.6	0.4	10.4	13	18	13
Dorsal kineties, number	3.0	0.0	0.0	0.0	3	3	20
	3.0	0.0	0.0	0.0	3	3	13
Caudal cirri, number	3.0	0.0	0.0	0.0	3	3	20
	3.0	0.0	0.0	0.0	3	3	13

[a]Data based on protargol-impregnated specimens.
CV = coefficient of variation (%); Max = maximum; Mean = average; Min = minimum; n = number of individual investigated; SE = standard error; SD = standard deviation. All measurements in μm.
[b]Frontal cirri not included.
[c]Pretransverse ventral and transverse cirri not included.

ties and additional two dikineties, dorsal cilia 3-5 μm long *in vivo*; caudal cirri lacking (Table 1; Figs. 1B, C, 2G, H).

Material examined. Mokpo Harbor (salinity, 21‰; 34º 47′N, 126º23′E), collected by Kyung-Min Park in June 2011. Yongyu-dong, Incheon (salinity, 30‰; 37°26′N 126°24′E), collected by Kang-San Kim in April, 2015.

Deposition. protargol-impregnated specimens (NIBR No. NIBRPR0000104109, NIBRPR0000104110) have been deposited in the National Institute of Biological Resources, Korea.

Habitat. Marine.

Distribution. China and Korea.

Remarks. There are six species in this genus: *Metaurostylopsis marina* Song *et al*., 2001; *M. rubra* Song & Wilbert, 2002; *M. salina* Lei *et al*., 2005; *M. struederkypkeae* Shao *et al*., 2008; *M. cheni* Chen *et al*., 2011; and *M. antarctica* Jung *et al*., 2011. *Metaurostylopsis marina* is the type species (Song *et al*., 2001; Berger, 2006; Shao *et al*., 2008; Chen *et al*., 2011; Jung *et al*., 2011). We used the morphometric data of the Mokpo population to make comparisons with congeners.

Metaurostylopsis cheni differs from *M. struederkypkeae* in the color of the cortical granules (yellowish to greenish vs. reddish) and in the number of cilia (3-5 vs. 4-8 in frontoterminal cirri; 7-11 vs. 12-20 in midventral pairs; 3-5 vs. 6-9 cirri in midventral row; 3-4 vs. 5-7 in left marginal rows) (Table 1) (Shao *et al*., 2008).

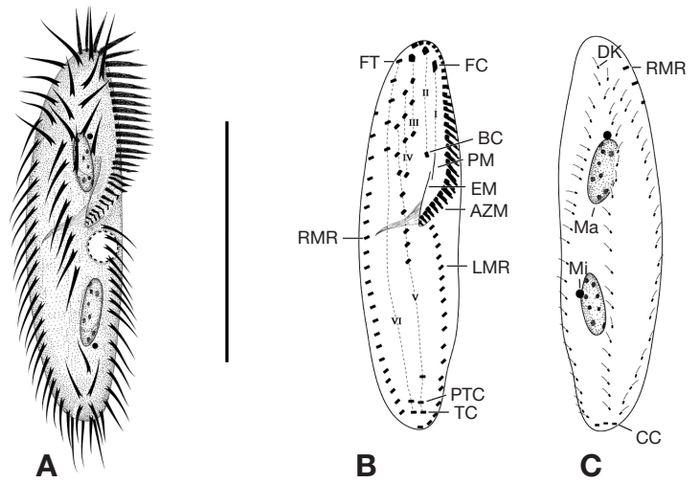


Fig. 3. Morphology of *Gonostomum strenuum* drawn from life (A) and after protargol impregnation (B, C). A, B, ventral views of representative individuals; C, dorsal view; AZM, adoral zone of membranelles; BC, buccal cirrus; CV, contractile vacuole; DK, dorsal kineties; EM, endoral membrane; FC, frontal cirri; FT, frontoterminal cirri; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronuclei; PM, paroral membrane; PTC, pretransverse cirri; RMR, right marginal row; TC, transverse cirri; CC, caudal cirri. Scale bar: 50 μm.

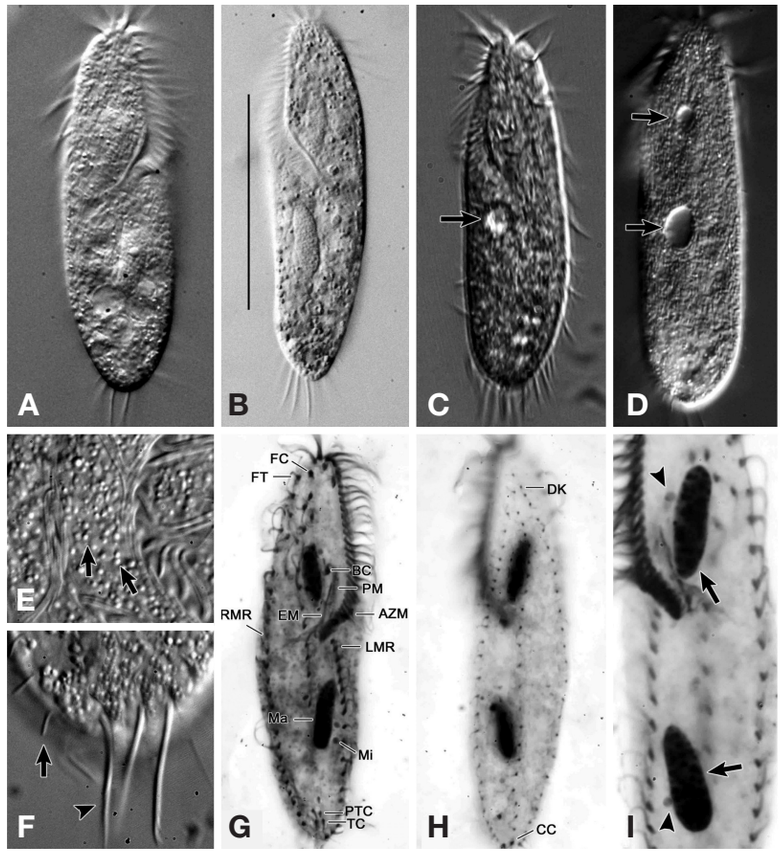


Fig. 4. Photomicrographs of *Gonostomum strenuum* from life (A-F) and after protargol impregnation (G-I). A, B, G, ventral views; C, D, H, dorsal views, arrows indicate contractile vacuole; E, arrows indicate cortical granules; F, dorsal cilia (arrow) and caudal cirri (arrowhead); I, macronuclear nodules (arrows) and micronuclei (arrowheads); AZM, adoral zone of membranelles, BC, buccal cirrus; DK, dorsal kineties; EM, endoral membrane; FC, frontal cirri; FT, frontoterminal cirri; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronuclei; PM, paroral membrane; PTC, pretransverse cirri; RMR, right marginal row; TC, transverse cirri; CC, caudal cirri. Scale bar: 50 μm.

Table 3. Morphometric data on *Lamtostyla granulifera* (upper line, Korean population from this study; lower line, Venezuelan population from Foissner, 1997).

Characteristics[a]	Mean	M	SD	SE	CV	Min	Max	n
Body length	111.0	110.0	15.0	3.4	13.5	90.0	130.0	20
	143.4	143.0	9.7	2.2	6.8	125.0	160.0	19
Body width	51.0	50.0	6.0	1.3	11.7	45.0	60.0	20
	64.5	65.0	9.0	2.1	14.0	49.0	80.0	19
Adoral membranelles length	29.7	30.0	1.8	0.4	6.2	26.0	32.0	20
	41.0	40	2.2	0.5	5.3	38.0	46.0	19
Adoral membranelles, number	23.3	24	1.2	0.3	5.2	21	25	20
	24.6	24	1.1	0.2	4.3	23	27	19
Macronuclear nodules, number	2.0	2	0.0	0.0	0.0	2	2	20
	2.0	2	0.0	0.0	0.0	2	2	19
Micronuclei, number	2.3	2	0.5	0.1	20.4	2	3	20
	2.1	2	–	–	–	1.0	3.0	19
Frontal cirri, number	3.0	3	0.0	0.0	0.0	3	3	20
	3.0	3	0.0	0.0	0.0	3	3	19
Buccal cirri, number	1.0	1	0.0	0.0	0.0	1	1	20
	1.0	1	0.0	0.0	0.0	1	1	19
Right ventral cirri, number	3.9	4	–	–	–	3	4	20
	4.0	4	0.0	0.0	0.0	4	4	19
Cirri left of ventral cirri, number	2.9	3	–	–	–	2	3	20
	3.0	3	0.0	0.0	0.0	3	3	19
Pretransverse ventral cirri, number	2.0	2	0	0	0	2	2	20
	1.9	2	–	–	–	1	2	19
Transvers cirri, number	5.0	5	0	0	0	5	5	20
	5.0	5	0	0	0	5	5	19
Right marginal cirri, number	37.9	38	2.3	0.5	6.0	34	42	20
	44.4	44	3.2	0.7	7.2	39	49	19
Left marginal cirri, number	35.2	35.5	1.4	0.3	4.1	33	38	20
	45.8	45	2.5	0.6	5.4	42	52	19
Dorsal kineties, number	3.0	3	0.0	0.0	0.0	3	3	20
	3.0	3	0.0	0.0	0.0	3	3	19

[a]Data based on protargol-impregnated specimens.
CV = coefficient of variation (%); Max = maximum; M = Median; Mean = average; Min = minimum; n = number of individual investigated; SE = standard error; SD = standard deviation. All measurements in μm.

Metaurostylopsis cheni and *M. antarctica* both have two types of cortical granules. However, *Metaurostylopsis cheni* can be separated from *M. antarctica* by the number of transverse cirri (5-8 vs. 2), left marginal rows (3-4 vs. 2), and right marginal rows (3-4 vs. 1) (Table 1) (Jung *et al.*, 2011).

Order Sporadotrichida Fauré-Fremiet, 1961
Family Gonostomatidae Small & Lynn, 1985
Genus *Gonostomum* Sterki, 1878

Gonostomum strenuum (Engelmann, 1862) Sterki, 1878 (Table 2; Figs. 3, 4)

Diagnosis. Cell size *in vivo* 85 × 25 μm on average, long ellipsoidal shape, slightly flexible (Figs. 3A, 4A). Adoral zone 40% of body length (Table 2; Fig. 4A). Contractile vacuole at left of mid-body (Fig. 4C). Cortical granules present; two elongated macronuclear nodules with two to four micronuclei (Table 2). Three frontal cirri and one buccal cirrus, two to three cirri in streak III (without frontal cirri), three to four cirri in streak IV, seven to nine cirri in streak V, four to six cirri in streak VI (without pretransverse cirri and transverse cirri), two to four pretransverse cirri, two transverse cirri, and one right and left marginal row (Table 2; Figs. 2B, 4G). Three bipolar dorsal kineties, dorsal cilia *in vivo* 3-4 μm long, with one caudal cirrus each (Table 2; Figs. 2C, 4F, H).

Material examined. Heuksan island, Jeollanam-do (34°39′N 125°24′E), collected by Jae-Ho Jung in November, 2011. Gangjeong-dong, Jeju-do province (33°14′ N, 126°30′E), collected by Kang-San Kim in March 2013.

Deposition. NIBR No. NIBRPR0000107086-NIBR PR0000107091 with protargol-impregnated specimens have been deposited in the National Institute of Biological Resources, Korea.

Habitat. Moss soil (pH 6).

Distribution. Germany, China, Spain, England, USA, Czechoslovakia, Mexico, Korea.

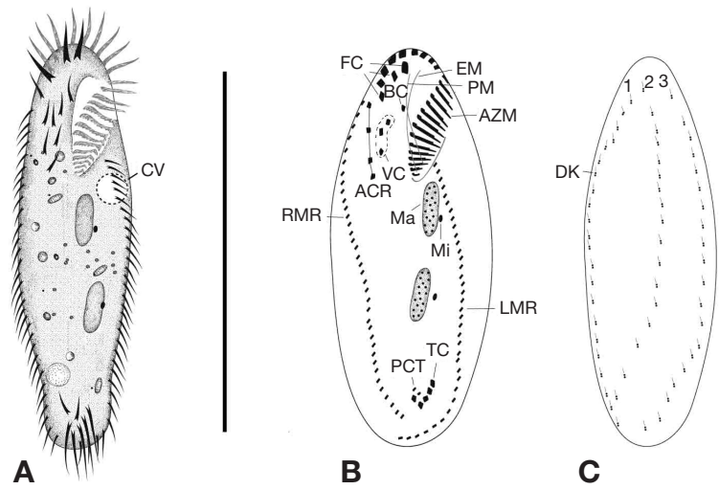


Fig. 5. Morphology of *Lamtostyla granulifera* drawn from life (A) and after protargol impregnation (B, C). A, B, ventral views; C, dorsal view; ACR, amphisiellid median cirral row; AZM, adoral zone of membranelles; BC, buccal cirrus; CV, contractile vacuole; DK, dorsal kineties; EM, endoral membrane; FC, frontal cirri; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronuclei; PM, paroral membrane; PTC, pretransverse cirri; RMR, right marginal row; TC, transverse cirri; VC, ventral cirri, left of ACR; 1-3, dorsal kineties 1-3. Scale bar: 100 μm.

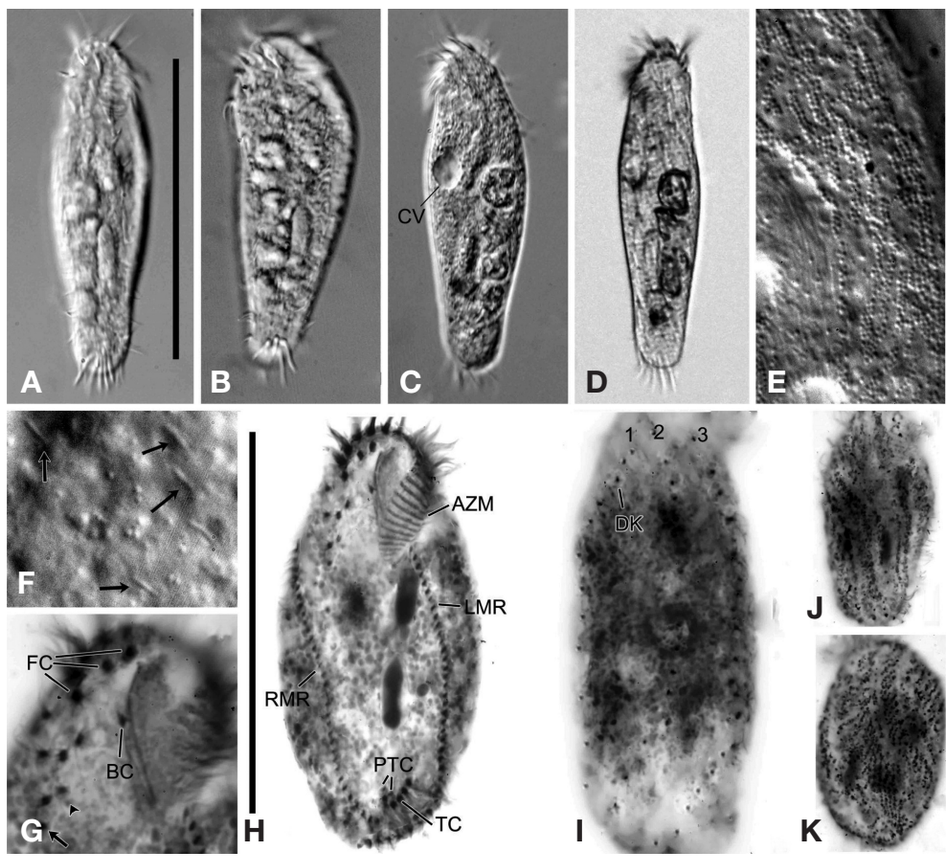


Fig. 6. Photomicrographs of *Lamtostyla granulifera* from life (A-F) and after protargol impregnation (G-K). A, B, E, G, H, J, ventral view of the specimen; C, D, F, I, K, dorsal views; E, distribution of cortical granules; F, arrows indicate dorsal cilia; G, anterior region of stained specimen, arrow marks amphisiellid median cirral row, arrowhead indicates ventral cirri; J, distribution of cortical granules on ventral surface; K, distribution of cortical granules on dorsal surface; AZM, adoral zone of membranelles; BC, buccal cirrus; DK, dorsal kineties; FC, frontal cirri; LMR, left marginal row; PTC, pretransverse cirri; RMR, right marginal row; TC, transverse cirri; 1-3, dorsal kineties 1-3. Scale bars: 100 μm.

Remarks. Most characteristics of the Korean population are shared with the Namibian population. The only varying feature is the micronuclei number (3-8 in the Namibian population vs. 2-4 in the Korean population) (Table 2; Foissner *et al*., 2002). The higher number of streak cirri and the different termination site of left marginal row (extending to the rear body margin) are distinguishing features of *G. strenuum* (Berger, 2011). In this study, all the Korean data were from the Jeju population.

Gonostomum strenuum can be separated clearly from *G. affine*, the type species of genus *Gonostomum*. *Gonostomum affine* differs from *G. strenuum* in the following characteristics: number of frontoterminal cirri (2 vs. 3-6); length of frontoventral row (short, row ends ahead of mid-body vs. long, row ends behind of mid-body); number of frontoventral cirri (6-15 vs. 20-25); existence of cortical granules (absent vs. present) (Table 2) (Berger, 2011).

Order Stichotrichida Fauré-Fremiet, 1961
Family Amphisiellidae Jankowski, 1979
Genus *Lamtostyla* Buitkamp, 1977

***Lamtostyla granulifera* Foissner, 1997 (Table 3; Figs. 5, 6)**

Diagnosis. Cell size *in vivo* 110 × 50 μm on average, ellipsoidal, highly flexible (Table 3; Fig. 6A, C). Adoral zone about 27% of body length (Table 3; Fig. 6A, I). Contractile vacuole at left and somewhat ahead of mid-body (Figs. 5A, 6C). Conspicuous cortical granules, colorless, 1-2 μm in diameter, arranged densely in rows on both cell surfaces (Fig. 6E, J, K). Two ellipsoidal macronuclear nodules with two to three micronuclei (Fig. 5B). Three frontal cirri; one buccal cirrus; four cirri in an amphisiellid median cirral row (ACR); three cirri left of the ACR; two pretransverse ventral cirri; and five enlarged transverse cirri (Figs. 5B, 6H). Three bipolar dorsal kineties, dorsal cilia *in vivo* 3-4 μm long; caudal cirri lacking (Figs. 5C, 6F, I).
Material examined. Obong-ri, Gangwon-do province (38°20′N 128°30′E) collected by Kang-San Kim in March, 2013.
Deposition. Protargol-impregnated specimens (NIBR No. NIBRPR0000107161, NIBRPR0000107162) have been deposited in the National Institute of Biological Resources, Korea.
Habitat. Soil with twigs under a tree (pH 5).
Distribution. Venezuela, Australia, Japan, Thailand, Cape Town (South Africa), Namibia, Korea.
Remarks. The genus *Lamtostyla* comprises three groups, i) the *Lamtostyla lamottei* group, ii) the *L. longa* group, and iii) the *L. granulifera* group (Berger, 2008). The *Lamtostyla lamottei* group can be distinguished from other two *Lamtostyla* groups by having more than four amphisiellid median cirral row (vs. four or fewer than four in the *L. granulifera* group, and the *L. longa* group) (Berger, 2008). The *Lamtostyla granulifera* group differs from the *L. longa* group in the number of dorsal kineties (3 vs. 5) (Berger, 2008).

Lamtostyla granulifera and *L. decorata*, which belong to the *L. granulifera* group, share many characteristics, including short amphisiellid median cirral row; cortical granules present; and number of frontal cirri, buccal cirrus, marginal cirral rows, dorsal kineties, and macronuclear nodules (Table 3) (Berger, 2008). *Lamtostyla granulifera* is however different from *L. decorata* in the following features: cortical granules (1-2 μm in diameter, densely distributed on both surfaces vs. 0.3 μm in diameter, packed around the dorsal bristles); body shape (broadly elliptical vs. slender and elongate, somewhat twisted); and ratio of adoral zone length (23-33% vs. 14-23%) (Berger, 2008).

The Korean population of *Lamtostyla granulifera* shows high similarity to the Venezuelan population, except in body length (90-130 μm in the Korean population vs. 125-160 μm in the Venezuelan population) and in the number of left marginal cirri (33-38 in the Korean population vs. 42-52 in the Venezuelan population (Table 3) (Berger, 2008).

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